

A novel method for preparation of tissue microarray

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Abstract

AIM: To improve the technique of tissue microarray (tissue chip).

METHODS: A new tissue microarraying method was invented with a common microscope installed with a special holing needle, a sampling needle, and a special box fixing paraffin blocks on the microscope slide carrier. With the movement of microscope tube and objective stage on vertical and cross dimensions respectively, the holing procedure on the recipient paraffin blocks and sampling procedure of core tissue biopsies taken from the donor blocks were performed with the refitted microscope on the same platform. The precise observation and localization of representative regions in the donor blocks were also performed with the microscope equipped with a stereoscope.

RESULTS: Highly-qualified tissue chips of colorectal tumors were produced by a new method, which simplified the conventional microarraying procedure, and was more convenient and accurate than that employing the existing tissue microarraying instruments.

CONCLUSION: Using the refitted common microscope to produce tissue microarray is a simple, reliable, cost-effective and well-applicable technique.

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INTRODUCTION

Tissue microarray (TMA, tissue chip) is one of the most important biochip technologies, following the gene chip and protein chip. This technology was first illustrated by Kononen *et al*^[1] in the magazine of *Nature Medicine* in 1998, who then has been working with the National Human Genome Research Institute. Illuminated by the idea of DNA microarray (gene

chip), Kononen *et al* proposed a new method of tissue microarray to improve the conventional process of immunostaining and fluorescent *in situ* hybridization (FISH) of individual tissue sections, which was laborious, low-efficient, vulnerable to various factors affecting the experiments and poor comparability.

TMA technology is sharply distinguished from the methods illustrated by Battifora *et al*^[2] (1986) and Wan *et al*^[3] (1987), which involved manually randomized rearrangement of tissue specimens. The difference lies in that the spots on TMA chips are minute (0.6 mm-2.0 mm in diameter), the quantity is huge (up to 1000 arrays at present), the shape is regular and the arrays are ordinal. The results obtained from TMAs are more scientific and comparable while the research is more informative, efficient and requires less consumption of reagents. Besides, it paves the way to the standardization of inspection and automation of analysis, which eventually makes the molecular pathological research step forward on the way of standardization and advancement in technology^[4-7].

Nevertheless, the current facilities of tissue microarrays are not advanced as expected but are costly. Moreover, it is not convenient to observe and locate the exact sites in the donor tissue blocks, or to install, adjust and replace the holing and sampling needles. Thus, the whole procedure takes much time that restricts the wide application of this particular technology^[8,9]. To make up the inadequacy, we proposed a new methodology of TMA in the following section, which is convenient, up-to-date and well applicable.

MATERIALS AND METHODS

Technical design

The key techniques of TMA included high-adhesive glass slide preparation, reliable tissue specimen acquisition, tissue core arrangement, fine sectioning and section transferring techniques, as well as tissue staining and molecular pathological technology. The core technology was the acquisition of reliable tissue specimen and arrangement of tissue cores, which involved tissue fixation, collection, dehydration, embedding, tissue array design and sampling procedures, especially the exact location of needed tissue, instruments and methods of sampling and puncture, *etc.* To avoid the inadequacy of the current TMA method, we proposed a new method for fabrication of TMAs through re-equipping the common microscope. With the precise mechanical controls and sampling site observation of the microscope, the new procedure simplified the holing, sampling and inserting process, increased reliabilities, and reduced costs and was ready for wide application.

Equipments and articles

Following equipments and articles were required: a refitted common light microscope; a set of holing, sampling needles and related components (China patent No. 03113734.2), which were installed in the objective holde; a special paraffin-fixing box (China patent No. 03113733.4) placed on the slide carrier; turn buckles and screw nuts fitted by the microscope pillar for controlling the moving range of object stage or the lens; the buckles for fixing glass slide carrier; sufficient paraffin-

embedded donor and recipient blocks; a temperature controlling oven; adhesive-coated glass slides; a tissue sectioning and transferring system as well as instruments, equipments and reagents of routine staining and molecular pathology. A stereomicroscope, a scanning apparatus and a computer automation analysis system were needed if available.

Tissue sample acquisition

This method could be applied in fabrication of paraffin embedded tissue microarrays. We took surgically or endoscopically sectioned fresh tissue, and banked paraffin-embedded tissue blocks as the specimen. The acquisition procedure included tissue fixation, collection, dehydration, hyaline and paraffin embedding, which were basically not different from the routine ones. However, to ensure the quality of TMA, procedures such as the use of fixation solutions and fixing time should be standardized and mRNA in the specimen should be preserved as much as possible.

Paraffin TMA block construction

This was one of the key techniques in the new procedure. Illustrated in Figure 1(A, B, C, D), the basic process included holing in the recipient blocks, location on the donor blocks, tissue core sampling and inserting. A. Holing A recipient block is fixed in the paraffin-fixing box, and the holing needle is tuned to the working place, and punched into the exact site of the recipient block and then is drawn back with adjustment of the rotating screw. B. Location A donor block is fixed in the paraffin-fixing box, and the stereomicroscope len is tuned to the working place, then the exact sampling site is observed and fixed in the center part of the microscopic vision field. C. Sampling The sampling needle is tuned to the working place, and inserted into the selected site of donor block, and retrieved with the adjustment of rotating screw. D. Inserting With the microscope stage moving, the recipient block moves exactly above the sampling needle. When the sampling needle moves exactly above the recipient hole, the core needle is pressed to squeeze out the tissue core and then inserted it into the recipient hole.

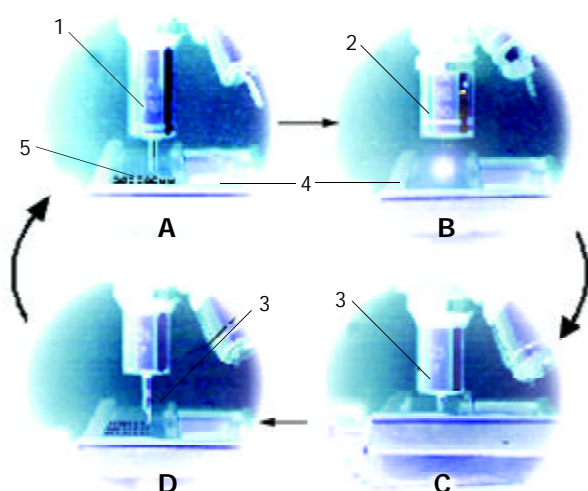


Figure 1 Procedures of paraffin-embed tissue micro-arraying. (1. holing needle, 2. stereomicroscope lens, 3. sampling needle, 4. paraffin block-fixing box, 5. recipient paraffin block).

Sectioning, transferring and staining or molecular pathological detecting

After insertion of all the samples, the recipient block was taken out from the fixing box and heated up to 58-65°C and the tissue

core was flattened by a glass slide and then cooled down. The next procedures were sectioning, tissue slice transferring and routine staining (HE) or PCR, immunofluorescent *in situ* hybridization, etc. (Figures 3-4).

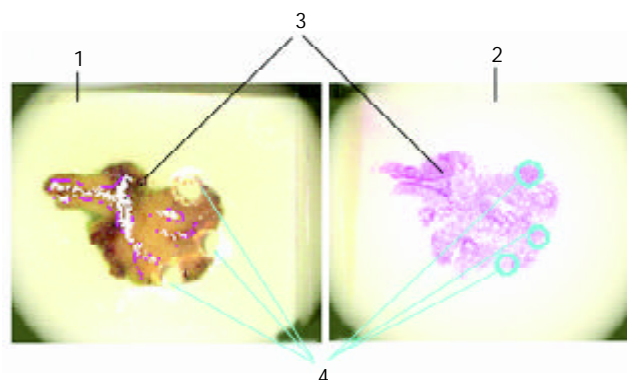


Figure 2 Defining accurate sampling sites in donor block under stereomicroscope. (1. Paraffin donor block and paraffin embedded tissue under stereomicroscope, 2. Routine section and HE staining of paraffin embedded donor tissue under stereomicroscope, 3. Regions of stroma, less cells or areas with no tissue, 4. Sampling locus.).

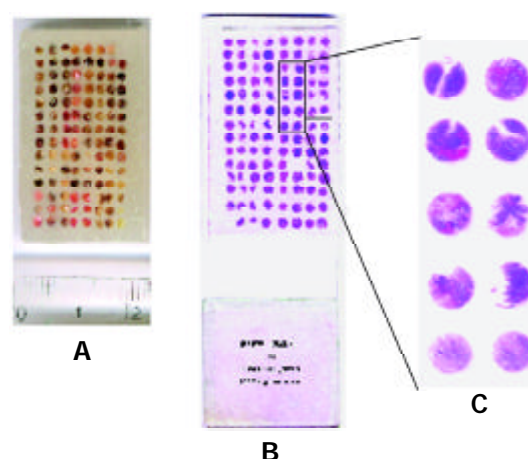


Figure 3 Pictures of paraffin-embedded tissue core arrangement (A), tissue spot arrays on slide (B) and magnified (X8) picture of selected array spots (C).

Computer imaging scan and data analysis

High-throughput digital imaging scanning and processing systems, as well as computer database programs such as Access, Excel were used to examine each core under microscope simultaneously. Data were analyzed with clinical information.

RESULTS

Employing the above method, high-quality tissue chips of colorectal tumors were successfully produced. Figure 3A is an image of the paraffin embedded tissue core arrays, which was 24 mm×35 mm in size. Every 3 core-specimens were obtained from each paraffin-embedded biopsy specimen, resulting in 111 cores from 35 colorectal tumor biopsy specimens (including colorectal adenocarcinoma, adenoma, non-adenoma polyps) and 2 normal colorectal tissue specimens. Each core was 1.3 mm in diameter and 0.7 mm in spacing.

The tissue micro-array of colorectal tumors on a glass slide (HE staining) is displayed in Figure 3B. The blank spot (dot array) on the left-hand corner was the location mark, and the blank spot in the center part was lost during sectioning and

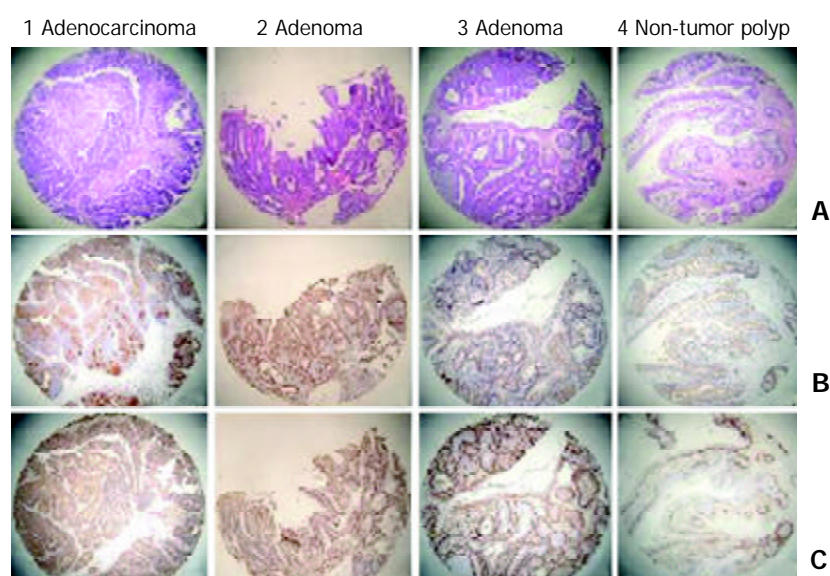


Figure 4 Photographs of different tissue array elements stained with HE(A) and immunohistochemistry (B: P53, C: PCNA).

Table 1 Comparison of gene, protein and tissue chips

	Gene chip	Protein chip	Tissue chip
Substrate	Definitive DNA or RNA (Oligo or cDNA)	Definitive proteins (antigens\ antibodies, etc.)	Many tissue samples
Probes	Labelled DNA or RNA (Cy3 and Cy5 -tyramide)	Labelled antigens or antibodies	Specific antigens or antibodies, as well as labelled DNA or RNA sequences
Target material	Various sequences of DNA or RNA	Multiple kinds of proteins	Morphology and DNA, RNA and proteins
Superiorities	Analyzing numerous genes simultaneously in the same sample	Analyzing numerous proteins simultaneously in the same sample	Profile of genes or proteins in numerous tissues or populations
Applications	Large-scale, parallel analysis of genetic alterations, functions and drug researches at DNA and RNA level	Large-scale, parallel analysis of concentrations, functional activities or interactions of proteins	Large-scale, parallel <i>in situ</i> analysis of DNA, RNA and proteins associated with clinical endpoints in hundreds of cell types or tissues at one time

staining, which made a rate of 0.8% non-evaluable core biopsy sections. The selected spots in Figure 3B were magnified (X8) in Figure 3C under a stereoscope and the dot arrays were in good order, sharp bordered, not crushed or deformed. All the arraying tissue specimens were representative of the interest regions, which coincidentally met the research demands.

Good staining quality was also attained on the same array sites in different slides stained with HE, immunohistochemistry and others respectively. The results were more comparable and sensitive on the chip slide as shown in Figure 4. Further detailed analyses would be reported soon.

DISCUSSION

As one of the three most important biochip technologies, tissue microarray has been accepted as a large-scale, parallel molecular analysis just like gene and protein chips^[3-6,10-15]. The results were more scientific, comparable, informative and efficient, and it needed less consumption of reagents^[16-23]. The difference lies in that the dot arrays of tissue chip were specimens from hundreds of different tissues and could show the features of morphology and expressions of DNA, RNA or proteins *in situ* associated with clinical endpoints at the same time. And the procedure was less complex and the results were more clinically applicable. Thus TMA is more feasible in the functional researches of gene and protein, especially in researches of gene or protein profiles in different tissues or populations. Therefore TMA has become one of the most

important methods in functional genomic and proteomic researches in the post-genomic era and, it has been expected to have wide applications in molecular pathology, drug discovery, monitoring of hygiene and environment, as well as national defense researches (Table 1)^[24,25].

However, the present technology of TMA is still not satisfactory. On the one hand, the instruments were not very advanced but costly. On the other hand, the present procedure took time and was not convenient to observe and locate the exact sites in donor tissue blocks^[8,9,26-32]. The new method to produce tissue microarray with refitted common microscope we presented here has the following advantages. (1) With the precise mechanical control of common microscope, the holing and sampling procedures in the paraffin recipient block and donor block are simpler and more accurate. (2) The punching procedure of holing and sampling needles is controlled by the adjustment of microscope stage or lens regulating screw, which frees us from direct finger operation and avoids needle gliding or trembling. This method is less laborious and easier and more accurate to control the holing and sampling depth. (3) The working place of holing and sampling needles and object lens changes with the rotation of objective holde and thus is easier to replace the needles and more convenient to apply the single-use holing and sampling needles in order to maintain a higher accuracy. (4) With this new method, the sampling sites can be easily and accurately observed and located under a microscope or a stereomicroscope and thus spares the efforts of marking the regions to punch on the face of the donor block and without

eyeballing, as is the case with the Beecher's instruments. (5) The whole process of holing, locating and sampling is performed with the same instrument on the same platform and thus spares uses of other platforms and instruments.

As TMA involves a number of related technologies and sciences, including engineering, surface physics and chemistry, pathology, molecular biology and information science, to produce high-quality tissue biochips, consideration should be also taken into the design of arrays, the qualities of holing and sampling needles, sectioning and transferring techniques, staining and other molecular pathological techniques. To improve the density and precision of arrays, the needles should be more minute in diameter and sharper in point. The arrangement of arrays should be in accordance with the research objectives and the computer imaging, and statistical tools should be provided for efficient management of a large amount of data generated from this high-throughput approach^[33-35].

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